Reprinted from Science, February 25, 1966, Vol. 151, No. 3713, pages 986-988





# Direct Evidence for the Cathodic Depolarization Theory of Bacterial Corrosion

Abstract. *Cathodic depolarization of mild steel by* Desulfovibrio desulfuricans *was demonstrated with benzyl viologen used as an electron acceptor. Direct measurement of the cathodic depolarization current indicated a maximum current density of 1 microampere per square centimeter. Aluminum alloys were also cathodically depolarized by the organism..*

In 1934, von Wolzogen Kühr and van der Vlugt proposed a theory for the anaerobic corrosion of iron by bacteria (*1*). In brief, the theory states that bacteria, primarily those of the genus *Desulfovibrio*, remove hydrogen that accumulates on the surface of iron as a result of their hydrogenase activity and reduce sulfate, yielding hydrogen sulfide. The electrons removed as a result of hydrogen utilization permit more iron to be dissolved or corroded at the anode. The hydrogen sulfide and the hydroxyl ions combine with the ferrous ions to form secondary reaction products at the anode.

Over the years evidence for and against this theory has accumulated. Much of this earlier evidence has been reviewed by Starkey (*2*). More recently, Raifsnider (*3*) and Scott (*4*) have presented observations that are not in agreement with this classical theory. Booth and his associates (*5*), using polarization techniques and weight loss measurements versus hydrogenase activity, have presented evidence for the theory. These methods, although involving standard techniques, are nevertheless indirect approaches and may be subject to various interpretations. The key step in the theory is the removal of hydrogen or electrons at the cathode and the subsequent dissolution of iron at the anode. If the *Desulfovibrio* cells are able to remove hydrogen or electrons from the surface of iron via an electron acceptor (sulfate), a deficiency of electrons should be created at places where there are no cells (anodes). As a result, the equilibrium $Fe \rightarrow Fe^{++} + 2e$ is upset and the reaction should be displaced to the right, causing more $Fe^{++}$ ions to go into solution (corrode).

Direct evidence for this step was obtained by substituting the dye, benzyl viologen, colorless when oxidized and violet when reduced (*6*), for sulfate as the electron acceptor to avoid the complicating factor of $H_2S$ reaction with the iron and to make the reduction process visible.

Through use of the medium, trypticase soy broth (Baltimore Biological Laboratory) plus 2 percent agar, it has been possible to culture *Desulfovibrio* readily and in pure culture on the surface of agar plates under a hydrogen atmosphere. Cells of *Desulfovibrio desulfuricans* (Mid-Continent Strain A), possessing hydrogenase activity, were grown on the surface of this medium, removed with a bacteriological loop, and placed on a small area on the surface of solidified washed (Noble) agar (2 percent) containing 0.01*M* tris buffer [tris (hydroxy methyl) aminomethane] and benzyl viologen (0.01 percent). This medium was adjusted to *p*H 7.0 ± 1 with HCl and autoclaved at 15 lb (6.8 kg) for 15 minutes. Polished (emery cloth), degreased (acetone), and sterilized (alcohol plus flame) coupons of 1010 mild steel (8 by 15 mm) were placed on the surface of the agar plates (Fig. 1) with one end on the area with the cells. The plates with the coupons were placed in a jar suitable for evacuation (Brewer's jar, Baltimore Biological Laboratories), and the atmosphere was replaced by nitrogen.

After about 17 to 24 hours in a temperature of 27° ± 1°C, the plates and coupons were removed. A dark violet area of reduced benzyl viologen was observed in the agar underneath the part of the coupon over the area previousiy covered with cells (Fig. 2*a*). Lighter areas of reduced benzyl viologen were observed in the agar underneath both ends of the coupon not in contact with the cells. These lightly reduced areas, probably due to the direct reduction of the dye by the metal, disappeared (oxidized) and left only the heavily reduced area produced by cellular reduction (Fig. 2*b*). A yellow-brown

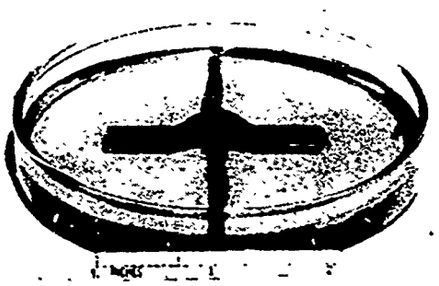


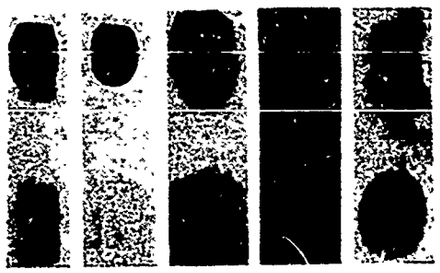
Fig. 1. Petri plate with 1010 steel coupon resting on agar surface.

area can be observed on the surface of the agar surrounding the areas that were in contact with the coupon. This material appears to be an insoluble iron compound, probably $Fe_2O_3$ or $Fe(OH)_3$, which was formed as a result of iron oxidation by small traces of oxygen present in the nitrogen, the agar, or the metal surface.

The iron in the agar was made visible (developed) by adding either potassium thiocyanate (for $Fe^{+++}$) or potassium ferricyanide (for $Fe^{++}$ or $Fe^{+++}$) with or without acid (HCl). In Fig. 2*c* equal portions of an aqueous 1-percent solution of potassium thiocyanate and HCl (10 percent concentrated, vol/vol) were added to the agar surface. Heavy concentrations of iron were indicated within several minutes where the insoluble iron "edge" was located. Since this iron was located at the surface, the iron thiocyanate complex was washed away by adding more of the reagents. The area of reduced benzyl viologen was still visible. After about 20 minutes, only the pink iron thiocyanate complex in the agar that had slowly developed ($Fe^{++}$ slowly oxidized to $Fe^{+++}$) remained visible (Fig. 2*d*). The reduced benzyl viologen had by this time been oxidized (colorless). Within 30 to 45 minutes, the pink iron thiocyanate complex began fading and eventually completely disappeared.

The use of nonacidic potassium ferricyanide (10-percent aqueous solution) is much more satisfactory because the color complex does not fade, but it must be added immediately to the agar surface after removing the plate from the nitrogen atmosphere to detect the $Fe^{++}$ ions. A plate developed with ferricyanide is shown (Fig. 2*e*) that indicates a heavy concentration of $Fe^{++}$ ions under the coupon not in contact with the cells (anode) and relatively few $Fe^{++}$ ions at the cathode (coupon in contact with the cells).

The results obtained seem to indicate that the steel coupon on the agar surface has corroded as a result of three reactions, which take up electrons or act as "electron sinks": (i) the combination of electrons with oxygen and water to form $(OH)^-$ ions, which react with the $Fe^{++}$ ions and more oxygen to form the "edge" effect; (ii) the direct uptake of electrons by oxidized benzyl viologen to form the violet reduced benzyl viologen; and (iii) the reduction of $H^+$ ions at the iron surface to form molecular or atomic hy-



a b c d e

Fig. 2. Areas in agar under coupon indicating location of reduced benzyl viologen and ferrous ions. (*a*) Agar surface immediately after removal of steel coupon. Dark area due to reduction of benzyl viologen by *Desulfovibrio* cells. (*b*) Same plate 10 minutes later, after the benzyl viologen that was reduced directly by the steel has been oxidized (decolorized) by exposure to the air. (*c*) A similar plate to which HCl (10-percent concentration in $H_2O$, vol/vol) and potassium thiocyanate (10 percent wt/vol) have been added. (*d*) Same plate as 2*c*, 20 minutes after addition of HCl and potassium thiocyanate. (*e*) Portion of plate that was developed with aqueous potassium ferricyanide (10 percent wt/vol) showing a heavy $Fe^{++}$ concentration at anode (no cells) and none at the cathode (area surrounded by black border of masking ink where a heavy concentration of cells was placed).

drogen, which is then removed. The electrons are utilized to reduce the benzyl viologen.

The iron that forms the "edge" can be distinguished from reactions ii and iii by omitting acid in the "development" process. The iron that forms in reaction ii (both anode and cathode) as distinguished from reaction iii may be detected by using control coupons (no cells in contact with either end of the coupon). By direct observation of the color intensity, it can be noted that the total quantity of $Fe^{++}$ ions evolved from both ends of the control coupon in reaction ii is much less than the total amount of iron evolved at the anode in reaction iii.

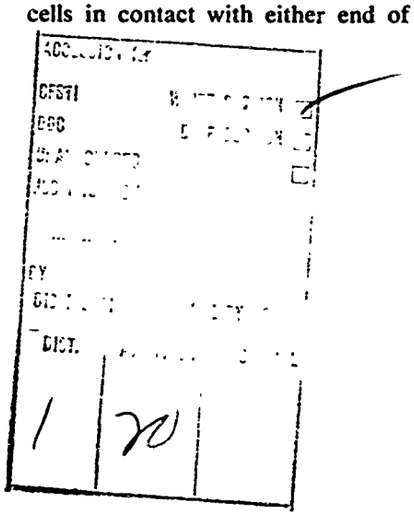
Since there was obviously a flow of electrons from the anode to the cathode, direct measurement of this cathodic depolarization current seemed possible. Two electrodes (each with a surface area of 1.1 $cm^2$), made from one of the coupons, were encased in lucite and secured by means of a holder that fit over a standard plastic petri dish bottom. After the two electrodes were dropped on the agar surface and secured, the petri dish was placed in a Brewer's jar and the air was replaced by nitrogen. The two electrodes were connected to a very sensitive vacuum tube voltmeter (Hewlett-Packard, model 412A) equipped with a recorder (Esterline-Angus, model AW).

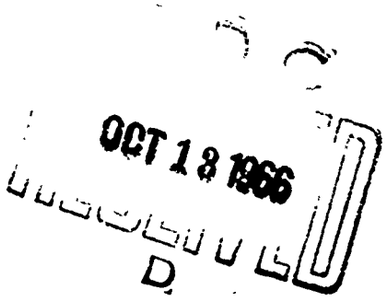
With a very large number of cells (entire surface growth of 3-day-old trypticase soy broth + agar plate) under one electrode (cathode) and none under the other electrode (anode), a sustained current density of about 1 $\mu a/cm^2$ was obtained for a period of about 9 hours. This corresponds to a corrosion rate of about 2.5 mdd (milligrams/$dm^2$ day) or about 0.00046 ipy (inches per year) with the formula mdd = ipy × 696 × density (*7*) and taking the density of 1010 steel as 7.85. No appreciable current was obtained in the absence of cells under the electrode. The electrode in contact with the cells (cathode) always showed a positive polarity and the anode a negative polarity, a standard dry cell being used as reference.

By use of this technique, it has also been demonstrated that aluminum and aluminum alloys can be cathodically depolarized. Metals more noble than iron and aluminum in the electromotive series, such as tin, zinc, and lead, appeared to be resistant to this type of attack. This may be due to the toxic effects of these metals and their ions on the hydrogen or other electron transport systems in the cells.

It thus appears that the mechanism proposed by the theory does indeed operate, if it can be assumed that sulfate acts in a similar fashion to benzyl viologen at the iron electrode as an electron acceptor. If such an assumption is made, the corrosion rate appears too small to account for the extensive corrosion (attributed to these organisms in nature) entirely by the Wolzogen Kühr theory.

WARREN P. IVERSON
*U.S. Army Biological Laboratories, Fort Detrick, Frederick, Maryland*




**References**

1. C. A. H. von Wolzogen Kühr and L. S. van der Vlugt, *Water* **18**, 147 (1934).
2. R. L. Starkey, *Producers Monthly* **22**, 12 (1958).
3. F. J. Raifsnider, 19th Annual Conference, National Association of Corrosion Engineers (1963).
4. W. R. Scott, *Mater. Protect.* **4**, 57 (1965).
5. G. H. Booth and A. K. Tiller, *Trans. Faraday Soc.* **56**, 1689 (1960); G. H. Booth and F. Wormwell, *1st International Congress on Metallic Corrosion* (Butterworths, London, 1961), p. 83; G. H. Booth and A. K. Tiller, *Trans. Faraday Soc.* **58**, 2510 (1962).
6. L. Michaelis and E. S. Hill, *J. Gen. Physiol.* **16**, 859 (1933).
7. H. H. Uhlig, *Corrosion and Corrosion Control* (Wiley, New York, 1953), pp. 39, 361.

17 January 1966